

BARKAN=2

U.S. APPLICATION NO. (If known) 37 CFR 1.37(b) P
09/403897FORM PTO-1300
(REV 11-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371INTERNATIONAL APPLICATION NO.
PCT/IL98/00196INTERNATIONAL FILING DATE
26 April 1998PRIORITY DATE CLAIMED
29 April 1997TITLE OF INVENTION
LEPTIN AS AN INHIBITOR OF TUMOR CELL PROLIFERATION

APPLICANT(S) FOR DO/EO/US

Dalit BARKAN et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (R/O/US).
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A **FIRST** preliminary amendment.
 - A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:
 1. A courtesy copy of the specification as originally filed.
 2. A courtesy copy of the first page of the International Publication (WO98/48831).
 3. A courtesy copy of the International Search Report.
 4. A courtesy copy of the International Preliminary Examination Report.
 5. Formal drawings, 9 sheets, figures 1-9.

17. The following fees are submitted:**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$970.00
 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00
 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$760.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00
 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =**CALCULATIONS PTO USE ONLY**

Surcharge of \$130.00 for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	27 - 20 =	7	X \$18.00
Independent claims	2 - 3 =		X \$78.00
"MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00

TOTAL OF ABOVE CALCULATIONS =

Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

SUBTOTAL =		\$ 840.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	+	\$

TOTAL NATIONAL FEE =

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

TOTAL FEES ENCLOSED =		\$ 840.00
Amount to be:	refunded	\$

charged \$

a. A check in the amount of \$ 840.00 to cover the above fees is enclosed.

b. Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4035. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO



SIGNATURE

Roger L. Browdy

NAME

25,618

REGISTRATION NUMBER

Date of this submission: October 28, 1999

09/403897

420 Rec'd PCT/PTO 28 OCT 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
Dalit BARKAN et al.)	
IA No.: PCT/IL98/00196)	
IA Filed: 26 April 1998)	Washington, D.C.
U.S. App. No.:)	
(Not Yet Assigned))	
National Filing Date:)	October 28, 1999
(Not Yet Received))	
For: LEPTIN AS AN INHIBITOR...)	Docket No.: BARKAN=2

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior
to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

--CROSS REFERENCE TO RELATED APPLICATION

The present application is the national stage under 35
U.S.C. 371 of PCT/IL98/00196, filed April 26, 1998. --

IN THE CLAIMS

Claim 3, line 1, delete "or claim 2".

Claim 4, line 1, delete "any one of claims 1-3", and
insert therefor --claim 1--.

Claim 6, line 1, delete "or claim 3".

Claim 7, line 1, delete "or claim 3".

Claim 8, line 1, delete "any one of claims 1-7", and
insert therefor --claim 1--.

Claim 9, line 1, delete "any one of claims 1-8", and
insert therefor --claim 1--.

Claim 12, line 1, delete "or claim 11".

Claim 13, line 1, delete "any one of claims 10-12", and
insert therefor --claim 10--.

Claim 15, line 1, delete "or claim 12".

Claim 16, line 1, delete "or claim 12".

Claim 17, line 1, delete "any one of claims 10-16", and
insert therefor --claim 10--.

Claim 18, line 1, delete "any one of claims 10-17", and
insert therefor --claim 10--.

Claim 19, line 1, delete "or claim 10".

Claim 21, line 1, delete "or 20".

Claim 22, line 1, delete "any one of claims 19-21", and
insert therefor --claim 19--.

Claim 23, line 1, delete "or 21".

Claim 24, line 1, delete "or 21".

Claim 25, line 1, delete "any one of claims 19-24", and
insert therefor --claim 19--.

Claim 26, line 1, delete "any one of claims 19-25", and
insert therefor --claim 19--.

Claim 27, line 3, delete "any one of claims 19-26", and
insert therefor --claim 19--.

REMARKS

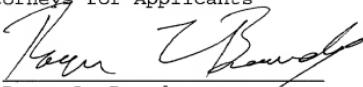
The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By



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LEPTIN AS AN INHIBITOR OF TUMOR CELL PROLIFERATION**FIELD OF THE INVENTION**

5 The present invention relates to leptin, a cytokine produced by adipocytes and affecting a variety of cells and tissues. More particularly, this invention relates to novel applications of leptin in the field of oncology.

BACKGROUND OF THE INVENTION

10 Leptin, an adipocyte-derived cytokine that regulates body weight, was identified by positional cloning of the murine *ob* gene (Zhang et al., 1994) and was shown to affect both food intake and thermogenesis (Campfield et al., 1995; Collins et al., 1996; Halaas et al., 1995; Pelleymounter et al., 1995; Weigle et al., 1995). High affinity leptin-binding sites were located in the choroid plexus; expression cloning of cDNA from this tissue provided the leptin receptor (OB-R) (Tartaglia et al., 1995). The known activities of leptin are mediated through its receptor in the hypothalamus. Leptin receptors are expressed, nonetheless, in additional organs, notably the kidney, lung and liver (Cioffi et al., 1996; Lee et al., 1996; Tartaglia et al., 1995). Furthermore, a different repertoire of leptin receptor-splice variants, differing in their cytoplasmic domain, is expressed in a tissue-specific manner in the mouse (Lee et al., 1996).

15 20 Therefore, in addition to control of food intake and body heat, leptin may exert other physiological functions.

Although leptin is produced by adipocytes, the recent finding of a correlation between excess fat and high levels of leptin in the serum was in contrast with the notion that leptin reduces food intake and body weight (Considine et al., 1996; Frederich et al., 1995; Lonnqvist et al., 1995; Maffei et al., 1995). This correlation, and the well established linkage between obesity and insulin resistance (Felber and Golay, 1995), suggested that leptin may modulate insulin-regulated responses. Indeed, it was recently reported that leptin reduced significantly the basal and insulin-induced tyrosine-phosphorylation of the insulin receptor substrate-1 (IRS-1). This effect of leptin on IRS-1 phosphorylation was specific, since tyrosine-phosphorylation of the insulin receptor (IR) β -chain was not reduced (Cohen et al., 1996).

Tyrosine-phosphorylation of IRS-1 by the IR kinase is a key step in the insulin receptor signaling cascade, leading to many of the known insulin activities (Araki et al., 1994; Cheatham and Kahn, 1995; Myers et al., 1994; Myers et al., 1994; Myers and White, 1993; Rose et al., 1994; Tamemoto et al., 1994; White and Kahn, 1994). Downstream signaling of 5 IRS-1 is mediated by several associated proteins, one of which is the Growth-factor Receptor-associated Binding protein-2 (GRB2) (Cheatham and Kahn, 1995).

The insulin receptor (IR) is regarded as a metabolic receptor, mediating the effects of insulin on glucose homeostasis. As such it is expressed on terminally differentiated tissues such as adipose tissue, liver and muscle. However, many studies have shown that IR is a potent mitogenic receptor *in vitro* and *in vivo* when expressed in tumor cells. For instance, functional IRs were identified in several breast cancer cell lines, as determined by tyrosine-phosphorylation of the IR in response to insulin treatment. Furthermore, the IR mediates a mitogenic response in these cells, as determined by [³H]thymidine incorporation (Milazzo et al., 1997; Millazzo et al., 1992).

15 Heretofore there has not been described the use of leptin in the field of oncology, in general; and in particular, leptin has not been described as being useful for inhibiting the proliferation of cells, especially proliferating cancer cells.

SUMMARY OF THE INVENTION

20 It is an object of the present invention to provide for the use of leptin as an inhibitor of cell proliferation, for example, to use leptin as an inhibitor of the proliferation of cancer cells.

It is another aim of the invention to provide for the use of leptin alone or in combination with other therapeutic agents for the treatment of various malignancies.

25 It is a further object of the present invention to provide for the use of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any one thereof, salts of any one thereof, and mixtures of any thereof, for the treatment of various malignancies.

It is yet another object of the present invention to provide pharmaceutical 30 compositions containing one or more of the above leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active

analogs or derivatives of any one thereof, and salts of any one thereof, for the treatment of various malignancies.

Other objects of the present invention will be set forth herein below or will be readily apparent from the following disclosure.

5 The present invention provides the use of leptin as an inhibitor of cell proliferation. Leptin may be useful, either alone or in combination with other therapeutic agents or approaches, for the treatment of various malignancies. A preferred embodiment of the invention is the use of leptin for the inhibition of human breast carcinoma cell proliferation. The proliferation of many types of tumor cells is increased in the presence of various growth factors such as insulin and IGF-I. The growth stimulatory effect of insulin and IGF-I on cells is mediated, at least in part, via the IRS-1/GRB2 pathway (Myers et al., 1993). This pathway is inhibited by leptin. Furthermore, IRS-1 is a substrate of receptor kinases of additional growth factors and cytokines, including IL-4 and IL-9 (Pernis et al., 1995; Yin et al., 1995; Yin et al., 1994). Therefore, leptin may inhibit the mitogenic responses of some or all of the aforementioned growth factors and cytokines, as well as other growth factors, thereby inhibiting the proliferation of a variety of tumor cells. Examples provided include inhibition of the IGF-I-induced proliferation and insulin-induced proliferation of the human breast cancer cell lines T-47D and MCF7. The present invention also provides the use of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, or active fragments or fractions of any one thereof, and salts of any one thereof as well as pharmaceutical compositions containing leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, or salts of any one thereof for the treatment of various malignancies.

20 More specifically, the present invention provides the use of an active agent selected from the group consisting of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any one thereof, salts of any one thereof, and mixtures of any thereof, as an inhibitor of tumor cell proliferation.

25 Embodiments of the above aspect of the present invention include :

30 (i) the use of the above active agent as an inhibitor of cell proliferation for the treatment of malignancies in mammals.

(ii) the use of the above active agent as an inhibitor of growth-factor dependent tumors.

(iii) the use of the above active agent as an inhibitor of human breast carcinoma cell proliferation.

5 (iv) the use of the above active agent for the treatment of human breast carcinomas.

(v) the use of the above active agent as an inhibitor of the growth stimulatory effect of insulin and IGF-I on tumor cells, as mediated, at least partially, by the insulin receptor substrate-1(IRS-1)/growth-factor receptor-associated binding protein-2 (GRB2) pathway.

10 (vi) the use of the above active agent as an inhibitor of the mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IL-4 and IL-9, for all of which IRS-1 is a substrate, for the treatment of tumors.

15 (vii) the use of the above active agent as an inhibitor of basal, IGF-I-induced and insulin-induced tumor cell proliferation for the treatment of human breast cancers.

(viii) the use of the above active agent wherein said active ingredient is leptin, and said leptin is used as said inhibitor or for said treatment.

Likewise, the present invention also provides for an active agent selected from the group consisting of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, 20 active fragments or fractions of any one thereof, active analogs or derivatives of any one thereof, salts of any one thereof, and mixtures of any thereof, for use in the preparation of a medicament for the inhibition of tumor cell proliferation.

Embodiments of this aspect of the invention include :

(i) an active agent as above for use in the preparation of a medicament for the 25 treatment of malignancies in mammals.

(ii) an active agent as above for use in the preparation of a medicament for the inhibition of growth-factor-dependent tumors.

(iii) an active agent as above for use in the preparation of a medicament for the inhibition of human breast carcinoma cell proliferation.

30 (iv) an active agent as above for use in the preparation of a medicament for the treatment of human breast carcinomas.

(v) an active agent as above for use in the preparation of a medicament for the inhibition of the growth stimulatory effect of IGF-I and insulin on tumor cells, as mediated, at least partially, by the IRS-1/GRB2 pathway.

5 (vi) an active agent as above for use in the preparation of a medicament for the inhibition of the mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IGF-I, IL-4 and IL-9, for all of which IRS-1 is a substrate, for the treatment of tumors.

(vii) an active agent as above for use in the preparation of a medicament for the inhibition of basal, IGF-I-induced and insulin-induced tumor cell proliferation, for the treatment of human breast cancers.

10 (viii) an active agent as above wherein said active agent is leptin, and said leptin is used for the preparation of said medicament.

Similarly, in another aspect, the present invention provides a pharmaceutical composition comprising as active ingredient an active agent as noted above and a pharmaceutically acceptable carrier, diluent or excipient, for the inhibition of tumor cell proliferation.

15 Embodiments of this aspect of the invention include :

(i) a pharmaceutical composition for the treatment of malignancies in mammals.

20 (ii) a pharmaceutical composition for the inhibition of growth-factor-dependent tumors.

(iii) a pharmaceutical composition for the inhibition of human breast carcinoma cell proliferation and thereby for the treatment of human breast carcinoma.

25 (iv) a pharmaceutical composition for the inhibition of the growth stimulatory effect of IGF-I and insulin on tumor cells, as mediated, at least partially, by the IRS-1/GRB2 pathway.

(v) a pharmaceutical composition for the inhibition of mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IL-4 and IL-9, for all of which IRS-1 is a substrate, and thereby for the treatment of tumors.

(vi) a pharmaceutical composition for the inhibition of basal, IGF-I-induced and insulin-induced tumor cell proliferation and thereby for the treatment of human breast cancers.

(vii) a pharmaceutical composition wherein said active ingredient is leptin.

The present invention also provides for a method for treating tumors in mammals or for inhibiting tumor cell proliferation in mammals comprising administering to a patient a pharmaceutical composition according to the invention as noted above in a suitable dosage form and by a suitable route of administration. Such dosage forms and routes of administration are usually determined by the professional practitioners following their examination of the patient.

Other aspects and embodiments of the present invention are set forth or will be readily apparent from the following detailed description of the invention.

DESCRIPTION OF THE FIGURES

Figure 1 shows the dependence of T-47D cell proliferation on insulin as determined by MTT staining.

Figure 2 shows the dependence of T-47D cell proliferation on IGF-I as determined by MTT staining.

Figure 3 shows the inhibition of insulin-induced T-47D cell proliferation in 10% fetal bovine serum (FBS) by murine leptin as determined by MTT staining.

Figure 4 shows the inhibition of insulin-induced T-47D cell proliferation in 2% FBS by murine leptin as determined by crystal violet staining.

Figure 5 shows the inhibition of IGF-I-induced T-47D cell proliferation in 10% FBS by murine leptin as determined by MTT staining.

Figure 6 shows the inhibition of IGF-I-induced T-47D cell proliferation in 2% FBS by murine leptin as determined by crystal violet staining.

Figure 7 shows the inhibition of IGF-I-induced T-47D cell proliferation in 2% FBS by
5 human leptin as determined by crystal violet staining.

Figure 8 shows the inhibition of insulin-induced MCF7 cell proliferation in serum-free
by murine leptin as determined by crystal violet staining.

10 Figure 9 shows the inhibition of IGF-I-induced MCF7 cell proliferation in serum-free
by murine leptin as determined by crystal violet staining.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns the use of leptin as an inhibitor of tumor cell proliferation. Typically, cell lines of human origin, derived from various tumors, may be grown in culture in the presence of growth medium supplemented with fetal bovine serum at a concentration of about 10% by volume. Cell proliferation under these conditions is defined hereinafter as "basal cell proliferation". Growth of many tumor cell lines is significantly enhanced when various growth factors such as insulin, epidermal growth factor or insulin-like
20 growth factor-I (IGF-I) are added to the above serum-supplemented culture medium. Inclusion of leptin in growth media at a range of concentrations from 3 to 600 nanomolar reduces both basal cell proliferation and growth-factor-dependent cell proliferation.

25 The results of the cell culture experiments given in the examples below indicate that leptin is useful for inhibiting the growth of various tumors. Hence, leptin may be useful for the treatment of various malignancies.

In a preferred embodiment of the present invention, leptin is used for the inhibition of breast cancer cell proliferation. When leptin is added to cultures of the human ductal breast carcinoma T-47D cells, (American Type Culture Collection, Rockville, MD; strain No ATCC HTB 133), their extent of proliferation is reduced. Similarly, when leptin is added to cultures

of the human breast adenocarcinoma MCF7 cells, (American Type Culture Collection, Rockville, MD; strain No. ATCC HTB 22), their extent of proliferation is reduced. Leptin inhibits both the basal, the IGF-I-induced and the insulin-induced proliferation of T-47D and MCF7 cells. Hence, leptin may be useful specifically for the treatment of breast carcinomas.

5 The growth stimulatory effect of insulin and IGF-I on cells is mediated, at least in part, via tyrosine phosphorylation of IRS-1 and subsequent association of IRS-1 with GRB2, leading to a mitogenic response. The anti-mitogenic effect of leptin may result from its ability to reduce the basal, the insulin-induced and the IGF-I-induced tyrosine phosphorylation of IRS-1, leading to reduced binding of GRB2 to IRS-1. Furthermore, IRS-1 is a substrate of
10 receptor kinases of other growth factors and cytokines, including IL-4 and IL-9 (Pernis et al., 1995; Yin et al., 1995; Yin et al., 1994). Therefore, leptin may inhibit the mitogenic responses of some or all of the aforementioned growth factors and cytokines, as well as other mitogens, thereby inhibiting the proliferation of a variety of tumor cells.

15 The present invention further relates to leptin derivatives and analogs, including leptin fusion proteins, leptin muteins, leptin receptor agonists, or active fragments or fractions thereof, and salts of all of same, and pharmaceutical compositions containing leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fractions thereof, or salts of all of same for the treatment of various cancers.

20 As used herein the term "muteins" refers to analogs of leptin, in which one or more of the amino acid residues are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the original sequence of leptin without changing considerably the activity of the resulting products as compared with wild type leptin or its active fragments or fractions. These muteins are prepared by known synthesis and/or by
25 site-directed mutagenesis techniques, or any other known technique suitable therefor.

Any such mutein preferably has a sequence of amino acids sufficiently duplicative of that of leptin such as to have substantially similar activity to leptin or its active fragments or fractions. Thus, it can be determined whether any given mutein has substantially the same activity as leptin by means of routine experimentation comprising subjecting such a mutein, e.g., to a simple cell proliferation assay, as a mutein which blocks cell proliferation retains
30 sufficient activity of leptin and therefore has at least one of the disclosed utilities of leptin and thus has substantially similar activity thereto.

In a preferred embodiment, any such mutein has at least 40% identity or homology with the sequence of one of the leptins. More preferably, it has at least 50%, at least 60%, at least 70%, at least 80% or, most preferably, at least 90% identity or homology thereto.

Muteins of leptin or its active fragments or fractions which can be used in accordance

5 with the present invention, or nucleic acid coding therefor, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz, G.E. et al., *Principles of Protein Structure*, Springer-Verlag, New
10 York, 1978; and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al, *supra*, at §§ A.1.1-A.1.24, and Sambrook et al, *Current Protocols in Molecular Biology*, Interscience N.Y. §§6.3 and 6.4 (1987, 1992), at Appendices C and D.

15 Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of leptin polypeptides or proteins or its active fragments or fractions may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the
20 molecule, Grantham, *Science*, Vol. 185, pp. 862-864 (1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e.g., under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g., cysteine residues, Anfinsen, "Principles That
25 Govern The Folding of Protein Chains", *Science*, Vol. 181, pp. 223-230 (1973). Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

30 Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

TABLE I Preferred Groups of Synonymous Amino Acids

	Amino Acid	Synonymous Group
	Ser	Ser, Thr, Gly, Asn
	Arg	Arg, Gln, Lys, Glu, His
5	Leu	Ile, Phe, Tyr, Met, Val, Leu
	Pro	Gly, Ala, Thr, Pro
	Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
	Ala	Gly, Thr, Pro, Ala
	Val	Met, Tyr, Phe, Ile, Leu, Val
10	Gly	Ala, Thr, Pro, Ser, Gly
	Ile	Met, Tyr, Phe, Val, Leu, Ile
	Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
	Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
	Cys	Ser, Thr, Cys
15	His	Glu, Lys, Gln, Thr, Arg, His
	Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
	Asn	Gln, Asp, Ser, Asn
	Lys	Glu, Gln, His, Arg, Lys
	Asp	Glu, Asn, Asp
20	Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
	Met	Phe, Ile, Val, Leu, Met
	Trp	Trp

TABLE II More Preferred Groups of Synonymous Amino Acids

	Amino Acid	Synonymous Group
5	Ser	Ser
	Arg	His, Lys, Arg
	Leu	Leu, Ile, Phe, Met
	Pro	Ala, Pro
	Thr	Thr
	Ala	Pro, Ala
	Val	Val, Met, Ile
	Gly	Gly
	Ile	Ile, Met, Phe, Val, Leu
	Phe	Met, Tyr, Ile, Leu, Phe
10	Tyr	Phe, Tyr
	Cys	Cys, Ser
	His	His, Gln, Arg
	Gln	Glu, Gln, His
	Asn	Asp, Asn
15	Lys	Lys, Arg
	Asp	Asp, Asn
	Glu	Glu, Gln
	Met	Met, Phe, Ile, Val, Leu
	Trp	Trp

TABLE III Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
5 Leu	Leu, Ile, Met
Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
10 Gly	Gly
Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
15 His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
20 Glu	Glu
Met	Met, Ile, Leu
Trp	Met

Examples of production of amino acid substitutions in proteins which can be used for obtaining muteins of leptin or its active fractions for use in the present invention include any known method steps, such as presented in US patents RE 33,653, 4,959,314, 25 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al., 4,965,195 to Namens et al; 4,879,111 to Chong et al; and 5,017,691 to Lee et al; and lysine substituted proteins presented in US patent No. 4,904,584 (Shaw et al).

30 In another preferred embodiment of the present invention, any mutein of leptin or its active fractions for use in the present invention has an amino acid sequence essentially

corresponding to that of leptin. The term "essentially corresponding to" is intended to comprehend proteins with minor changes to the sequence of the natural protein which do not affect the basic characteristics of the natural proteins, particularly insofar as its ability to inhibit cell proliferation is concerned. The type of changes which are generally considered to 5 fall within the "essentially corresponding to" language are those which would result from conventional mutagenesis techniques of the DNA encoding leptin, resulting in a few minor modifications, and screening for the desired activity in the manner discussed above.

Muteins in accordance with the present invention include proteins encoded by a nucleic acid, such as DNA or RNA, which hybridizes to DNA or RNA which encodes leptin in accordance with the present invention, under stringent conditions. Such nucleic acid would be a prime candidate to determine whether it encodes a polypeptide which retains the functional activity of leptin of the present invention. The term "stringent conditions" refers to hybridization and subsequent washing conditions which those of ordinary skill in the art conventionally refer to as "stringent". See Ausubel et al., Current Protocols in Molecular Biology, supra, Interscience, NY, §§6.3 and 6.4 (1987, 1992), and Sambrook et al., supra. Without limitation, examples of stringent conditions include washing conditions 12-20°C below the calculated T_m of the hybrid under study in, e.g., 2 x SSC and 0.5% SDS for 5 minutes, 2 x SSC and 0.1% SDS for 15 minutes; 0.1 x SSC and 0.5% SDS at 37°C for 30-60 minutes and then a 0.1 x SSC and 0.5% SDS at 68°C for 30-60 minutes. Those of ordinary 20 skill in this art understand that stringency conditions also depend on the length of the DNA sequences, oligonucleotide probes (such as 10-40 bases) or mixed oligonucleotide probes. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC. See Ausubel, supra.

The term "leptin fusion proteins" or simply "fused protein" refers to a 25 polypeptide comprising leptin or its active fractions or a mutein thereof, fused with another protein which, e.g., has an extended residence time in body fluids. Leptin or its active fractions may thus be fused to another protein, polypeptide or the like.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of leptin, its active fractions, muteins, or leptin fusion proteins thereof.

Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, 5 arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid. Of course, any such salts must have substantially similar activity to leptin or its active fractions.

"Functional derivatives" as used herein cover derivatives of leptin or its active fragments or fractions and its muteins and leptin fusion proteins, which may be prepared from 10 the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein which is substantially similar to the activity of leptin, and do not confer toxic properties on compositions containing it. These derivatives may, for example, include polyethylene glycol 15 side-chains which may mask antigenic sites and extend the residence of leptin or its active fractions in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl 20 moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fragments or fractions" of leptin, leptin muteins and leptin fusion proteins, the present invention covers any fragment or precursors of the polypeptide chain of leptin, or fused proteins containing any such fragment of leptin, alone or together with associated molecules or residues linked thereto, e.g., sugar or phosphate residues, or aggregates of any 25 of the above derivatives, provided said fraction has substantially similar activity to leptin.

The present invention further relates to use of natural and synthetic agonists of the leptin receptor, which are essentially similar to leptin in their ability to inhibit cell proliferation. Such agonists may be selected from a library of peptides, a library of peptide analogs or a random library of organic molecules. Selection is done by means known in the art, essentially 30 by the ability of the selected agonists to bind to the leptin receptor. For example, a library of random peptides may be prepared as prokaryotic expression plasmids carrying a DNA coding

for a random peptide, fused to a carrier protein. Another example is a phage display system in which the expression system is a phage containing DNA coding for a random peptide and incorporated into one of the external phage proteins. Phages coding for fused peptide agonists or antagonists are isolated from the phage library by e.g., panning over surfaces coated with 5 the leptin receptor. Bound phages are isolated and then amplified in bacteria. Several rounds of panning - amplification are usually required in order to obtain phages expressing fused peptides that have high affinity for the leptin receptor. The isolated phage is then amplified and the sequence of DNA coding for the peptide is determined. Alternatively, random peptide libraries or libraries of other molecules are prepared by solid phase synthesis on polymeric 10 beads by means known in the art. Beads carrying a peptide or other molecule having affinity for the leptin receptor are selected from the library, e.g., by binding of labeled leptin receptor, e.g., fluorescently labeled leptin receptor. Positive beads are then picked up and the structure 15 of the peptide or other molecule present on the bead is determined. If the bead carries a peptide, the peptide sequence is determined by protein sequence analysis. If the bead is a representative of a random library of organic molecules then the molecule is cleaved from the bead and its structure is determined by means known in the art, such as mass spectrometry, nuclear magnetic resonance and the like. Candidate peptides identified by their affinity for the leptin receptor are then further selected by their ability to inhibit cell proliferation in the aforementioned manner.

20 Accordingly, leptin, its active fractions, leptin muteins, leptin fusion proteins, leptin receptor agonists and their salts, functional derivatives, and active fragments or fractions thereof are indicated for the treatment of various malignancies, preferably for growth factor-dependent tumors and more preferably for breast carcinomas.

25 The present invention further relates to use of pharmaceutical compositions comprising a pharmaceutically acceptable carrier and leptin of the invention, or its active muteins, fused proteins, leptin receptor agonists and their salts, functional derivatives or active fractions thereof.

30 The pharmaceutical compositions of the invention are prepared for administration by mixing leptin or its derivatives, or leptin receptor agonists with physiologically acceptable carriers, and/or stabilizers and/or excipients, and prepared in dosage form, e.g., by lyophilization in dosage vials. The method of administration can be via any of the accepted

modes of administration for similar agents and will depend on the condition to be treated, e.g., intravenously, intramuscularly, subcutaneously, by local injection or topical application, or continuously by infusion, etc. The amount of active compound to be administered will depend on the route of administration, the disease to be treated and the condition of the patient. Local injection, for instance, will require a lower amount of the protein on a body weight basis than will intravenous infusion. Typical active amounts of leptin to be injected are 0.1-1000 microgram/kg body weight and preferably 1 to 10 micrograms/kg. Active amounts of leptin derivatives and leptin receptor agonists may be essentially the same as those of leptin on a molar basis.

Leptin may be administered to cancer patients, e.g., by injection, either alone or in combination with other therapeutic agents or in combination with other therapeutic approaches.

The invention will now be illustrated by the following non-limiting examples:

EXAMPLE 1: Determination of cell proliferation by MTT staining

Reagents:

MTT stock: (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide 5 mg/ml in phosphate-buffered saline. Stored at -20°C until used.

Solvent: Conc. HCl (450 microliters) in 2-propanol (100 ml).

Procedure:

Grow cells in 96 well plates in the presence of various growth stimulants and growth inhibitors. At a desired time add MTT stock (10 microliters) to each well. Incubate 2.5-3 hours at 37°C. Aspirate supernatant with vacuum using a fine gauge needle. Add Solvent (100 microliters) and read the absorbance with a microplate reader using a 570 nM filter with background subtraction at 630 nm.

EXAMPLE 2: Determination of cell proliferation by crystal violet staining**Procedure:**

Grow cells in 96 well plates in the presence of various growth stimulants and growth inhibitors. At the desired time add 12.5% glutaraldehyde (40 microliters) to each well.

5 Incubate 30 minutes at room temperature. The microplate was then washed with water, dried and aq. crystal violet (0.1% , 0.1 ml) was added to each well. The microplate was further incubated for 30 min., washed with water and read at 540 nm with background subtraction at 630 nm.

EXAMPLE 3: Determination of the insulin-dependent T-47D cell proliferation

Human T-47D cells (American Type Culture Collection, Rockville, MD; strain No. ATCC HTB 133), were seeded into 96 well plates at 3×10^5 cells/ml in DMEM and 10% fetal bovine serum (FBS), 0.1 ml per well. Human insulin was added to different wells at increasing concentrations, the plates were incubated for 72 hours and the number of cells was then determined by staining with MTT (Figure 1). The results are average of 8 replicates. Based on the extent of cell proliferation as shown in Figure 1, a concentration of 50 nM insulin was used for further studies.

EXAMPLE 4: Determination of the IGF-I-dependent T-47D cell proliferation

20 Human T-47D cells were seeded into 96 well plates at 3×10^5 cells/ml in DMEM and 10% FBS, 0.1 ml per well. Human IGF-I was added to different wells at increasing concentrations, the plates were incubated for 3 days and the number of cells was then determined by MTT staining (Figure 2). The results are average of 8 replicates. Based on the extent of cell proliferation as shown in Figure 2, a concentration of 50 ng/ml IGF-I was used

25 for further studies.

EXAMPLE 5: Inhibition of insulin-induced T-47D cell proliferation by leptin

T-47D cells (3×10^5 cells/ml) in DMEM supplemented with 10% FBS were seeded into 96 well plates (0.1 ml per well). Cells were treated with insulin (50 nM), with or without the

indicated concentrations of murine leptin. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with MTT. The data are mean ± standard error (SE, n=8). The results show that leptin inhibited significantly the insulin-induced cell proliferation (Figure 3).

5 T-47D cells (3x10⁵ cells/ml) in DMEM supplemented with 10% FBS were seeded into 96 well plates (0.1 ml per well). After one day the medium was replaced by DMEM supplemented with 2% FBS and after one day the cells were treated with insulin (50 nM), with or without the indicated concentrations of murine leptin in DMEM-2% FBS. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with crystal violet. The data are mean ± SE, (n=8). The results show that leptin inhibited significantly the insulin-induced cell proliferation (Figure 4).

EXAMPLE 6: Inhibition of IGF-I-induced T-47D cell proliferation by leptin

10 T-47D cells (3x10⁵ cells/ml) in DMEM supplemented with 10% FBS were seeded into 96 well plates (0.1 ml per well). Cells were treated with IGF-I (50 ng/ml), with or without the indicated concentrations of murine leptin. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with MTT. The data are mean ± standard error (SE, n=8). The results show that leptin inhibited significantly the IGF-I-induced cell proliferation (Figure 5).

20 T-47D cells (3x10⁵ cells/ml) in DMEM supplemented with 10% FBS were seeded into 96 well plates (0.1 ml per well). After one day the medium was replaced by DMEM supplemented with 2% FBS and after one day the cells were treated with IGF-I (50 ng/ml), with or without the indicated concentrations of murine leptin in DMEM-2% FBS. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with crystal violet. The data are mean ± standard error (SE, n=8). The results show that leptin inhibited significantly the IGF-I-induced cell proliferation (Figure 6).

25 T-47D cells (3x10⁵ cells/ml) in DMEM supplemented with 10% FBS were seeded into 96 well plates (0.1 ml per well). After one day the medium was replaced by DMEM supplemented with 2% FBS and after one day the cells were treated with IGF-I (50 ng/ml), with or without the indicated concentrations of human leptin in DMEM-2% FBS. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with crystal

violet. The data are mean \pm standard error (SE, n=8). The results show that leptin inhibited significantly the IGF-I-induced cell proliferation (**Figure 7**).

EXAMPLE 8: Inhibition of insulin-induced MCF7 cell proliferation by leptin

5 Human breast adenocarcinoma MCF7 cells (3×10^4 cells/ml, American Type Culture Collection, Rockville, MD; strain No. ATCC HTB 22) supplemented with 6% FBS were seeded into 96 well plates (0.1 ml per well). After one day the medium was replaced by serum-free DMEM and after one day the cells were treated with insulin (50 nM) with or without the indicated concentrations of murine leptin in a serum-free medium. The Plates were incubated at 37°C in 5% CO₂ for 48 hours. The cells were then stained with crystal violet. The data are mean \pm SE, (n=8). The results show that leptin inhibited significantly the insulin-induced cell proliferation (**Figure 8**).

EXAMPLE 9: Inhibition of IGF-I-induced MCF7 cell proliferation by leptin

15 Human breast adenocarcinoma MCF7 cells in DMEM supplemented with 10% FBS were seeded into 96 well plates (3×10^4 cells/ml, 0.1 ml per well). After one day the medium was replaced by a serum-free medium. After one day the cells were treated with IGF-I (50 ng/ml) with or without the indicated concentrations of murine leptin in a serum-free medium. The Plates were incubated at 37°C in 5% CO₂ for 96 hours. The cells were then stained with 20 crystal violet. The data are mean \pm SE, (n=8). The results show that leptin inhibited significantly the insulin-induced cell proliferation (**Figure 9**).

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CLAIMS

1. The use of an active agent selected from the group consisting of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any one thereof, salts of any one thereof, and mixtures of any thereof, as an inhibitor of tumor cell proliferation.
2. The use of an active agent according to claim 1 as an inhibitor of cell proliferation for the treatment of malignancies in mammals.
3. The use of an active agent according to claim 1 or claim 2 as an inhibitor of growth-factor dependent tumors.
4. The use of an active agent according to any one of claims 1-3, as an inhibitor of human breast carcinoma cell proliferation.
5. The use of an active agent according to claim 4 for the treatment of human breast carcinomas.
6. The use of an active agent according to claim 1 or claim 3 as an inhibitor of the growth stimulatory effect of insulin on tumor cells, as mediated, at least partially, by the insulin receptor substrate-1 (IRS-1)/growth-factor receptor-associated binding protein-2 (GRB2) pathway.
7. The use of an active agent according to claim 1 or claim 3 as an inhibitor of the mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IGF-1, IL-4 and IL-9, for all of which IRS-1 is a substrate, for the treatment of tumors.
8. The use of an active agent according to any one of claims 1-7 as an inhibitor of basal and insulin-induced tumor cell proliferation for the treatment of human breast cancers.

9. The use of an active agent according to any one of claims 1-8, wherein said active ingredient is leptin, and said leptin is used as said inhibitor or for said treatment.

5 10. An active agent selected from the group consisting of leptin, leptin fusion proteins, leptin muteins, leptin receptor agonists, active fragments or fractions of any one thereof, active analogs or derivatives of any one thereof, salts of any one thereof, and mixtures of any thereof, for use in the preparation of a medicament for the inhibition of tumor cell proliferation.

10 11. An active agent according to claim 10 for use in the preparation of a medicament for the treatment of malignancies in mammals.

15 12. An active agent according to claim 10 or claim 11 for use in the preparation of a medicament for the inhibition of growth-factor-dependent tumors.

13. An active agent according to any one of claims 10-12 for use in the preparation of a medicament for the inhibition of human breast carcinoma cell proliferation.

20 14. An active agent according to claim 13 for use in the preparation of a medicament for the treatment of human breast carcinomas.

25 15. An active agent according to claim 10 or claim 12 for use in the preparation of a medicament for the inhibition of the growth stimulatory effect of insulin on tumor cells, as mediated, at least partially, by the IRS-1/GRB2 pathway.

16. An active agent according to claim 10 or claim 12 for use in the preparation of a medicament for the inhibition of the mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IGF-1, IL-4 and IL-9, for all of which IRS-1 is a substrate, for the treatment of tumors.

17. An active agent according to any one of claims 10-16, for use in the preparation of a medicament for the inhibition of basal and insulin-induced tumor cell proliferation, for the treatment of human breast cancers.

5 18. An active agent according to any one of claims 10-17, wherein said active agent is leptin, and said leptin is used for the preparation of said medicament.

19. A pharmaceutical composition comprising as active ingredient an active agent according to claim 1 or claim 10 and a pharmaceutically acceptable carrier, diluent or excipient, for the inhibition of tumor cell proliferation.

10 20. A pharmaceutical composition according to claim 19 for the treatment of malignancies in mammals.

15 21. A pharmaceutical composition according to claim 19 or 20 for the inhibition of growth-factor-dependent tumors.

22. A pharmaceutical composition according to any one of claims 19-21 for the inhibition of human breast carcinoma cell proliferation and thereby for the treatment of human breast carcinoma.

20 23. A pharmaceutical composition according to claim 19 or 21 for the inhibition of the growth stimulatory effect of insulin on tumor cells, as mediated, at least partially, by the IRS-1/GRB2 pathway.

25 24. A pharmaceutical composition according to claim 19 or 21 for the inhibition of mitogenic responses in tumor cells to one or more receptor kinases, growth factors and cytokines of the group consisting of IGF-1, IL-4 and IL-9, for all of which IRS-1 is a substrate, and thereby for the treatment of tumors.

25. A pharmaceutical composition according to any one of claims 19-24 for the inhibition of basal and insulin-induced tumor cell proliferation and thereby for the treatment of human breast cancers.

5 26. A pharmaceutical composition according to any one of claims 19-25, wherein said active ingredient is leptin.

27. A method for treating tumors in mammals or for inhibiting tumor cell proliferation in mammals comprising administering to a patient a pharmaceutical composition according to any one of claims 19-26 in a suitable dosage form and by a suitable route of administration.

10

ABSTRACT

5 The use of leptin and leptin-related molecules in oncology is disclosed.

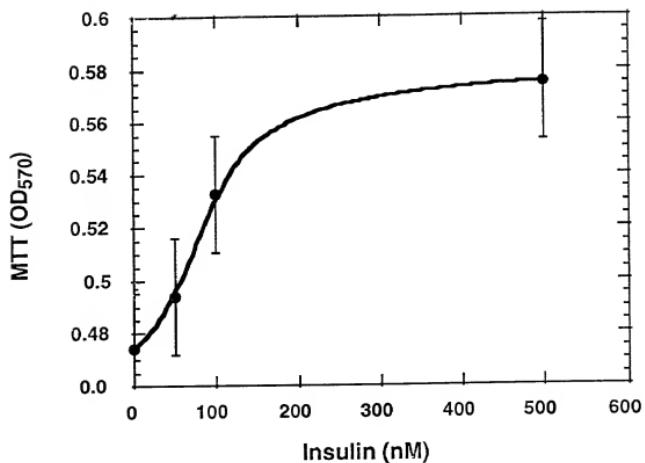


Figure 1

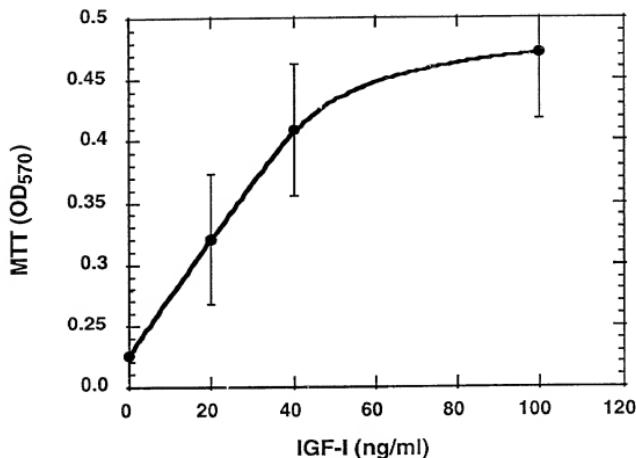


Figure 2

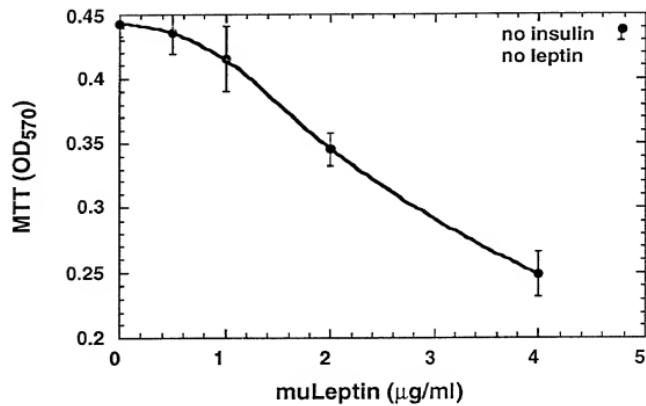


Figure 3

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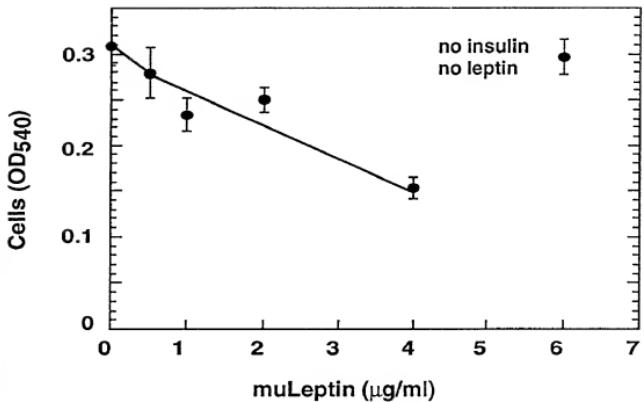


Figure 4

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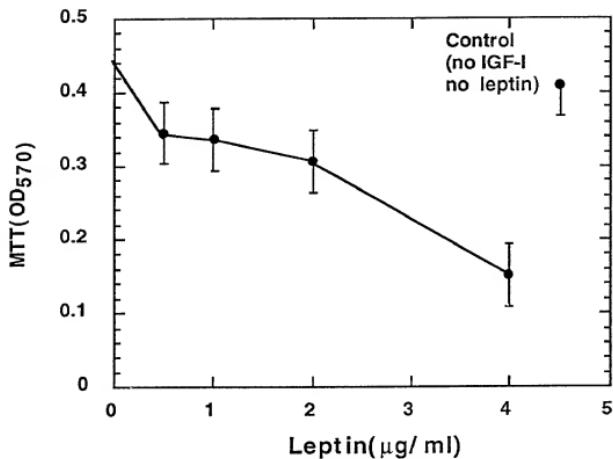


Figure 5

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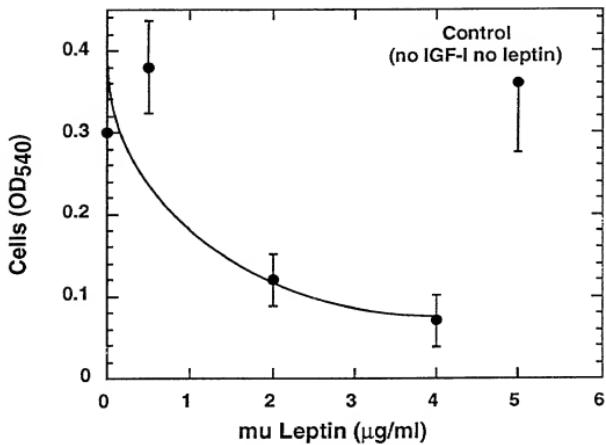


Figure 6

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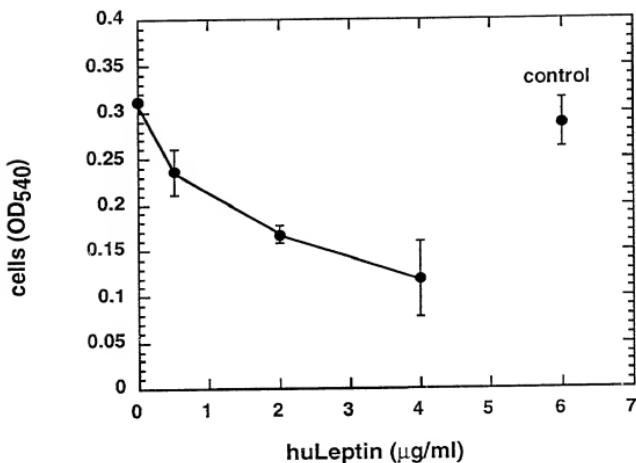
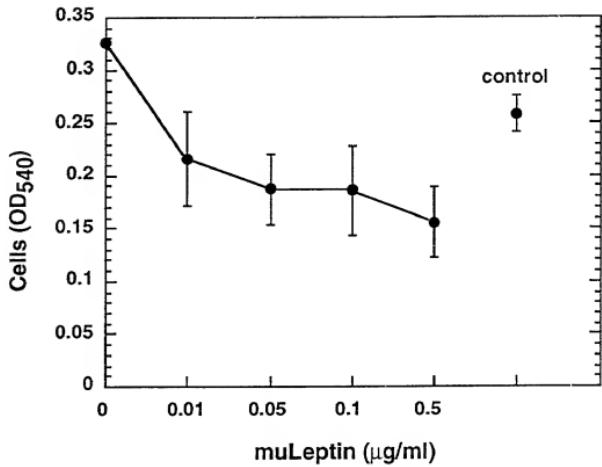


Figure 7

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**Figure 8**

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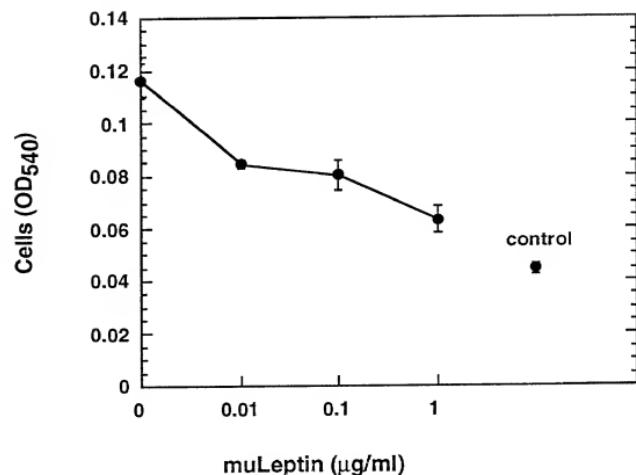


Figure 9

Page 1 of 2

Original Supplemental

Atty.Docket: BARKAN=2

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
LEPTIN AS AN INHIBITOR OF TUMOR CELL PROLIFERATION

LEPTIN AS AN INHIBITOR OF TUMOR CELL PROLIFERATION

the specification of which (check one)

[1] is attached hereto:

was filed in the United States under 35 U.S.C. §111 on _____, as
USSN *; or

[XXX] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/IL98/00196; filed April 26, 1998, entry requested on October 28, 1999 *: national stage application received

USSN _____ *; §371/§102(e) date _____ * (*if k
and was amended on October 28, 1999 (if applicable)

Include dates of amendments under PCT Art. 19 and 34 if PCT

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

120733 Israel 29 April 1997 (Number) (Country) (Day Month Year Filed) YES NO

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

SHERIDAN HEIMARK, REG. NO. 20,520. - ROGER L. BROUDY, REG. NO. 25,618 - ANNE M. KIRMBAU, REG. NO. 25,884
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The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from **INTER-LAB LTD.**, as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

Page 2 of 2

Atty.Docket: BARKAN=2

Title: LEPTIN AS AN INHIBITOR OF TUMOR CELL PROLIFERATION

U.S. Application filed _____, Serial No. _____

PCT Application filed 26 April 1998, Serial No. PCT/IL98/00196

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR <u>Dalit BARKAN</u>		INVENTOR'S SIGNATURE <u>Dalit Barkan</u>	DATE <u>x/1/feb/00</u>
RESIDENCE <u>Rehovot, Israel</u>	ILX		CITIZENSHIP <u>Israel</u>
POST OFFICE ADDRESS <u>Vilkomitch Street 22a, Rehovot 76448, Israel</u>			
FULL NAME OF SECOND JOINT INVENTOR <u>Batyra COHEN</u>		INVENTOR'S SIGNATURE <u>Batyra Cohen</u>	DATE <u>x/14/feb/00</u>
RESIDENCE <u>Tel Aviv, Israel</u>	ILX		CITIZENSHIP <u>Israel</u>
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FULL NAME OF THIRD JOINT INVENTOR <u>Menachem RUBINSTEIN</u>		INVENTOR'S SIGNATURE <u>Menachem Rubinstein</u>	DATE <u>x/14/feb/00</u>
RESIDENCE <u>Givat Shmuel, Israel</u>	ILX		CITIZENSHIP <u>Israel</u>
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FULL NAME OF FOURTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF FIFTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: BARKAN=2

In re Application of:)	Art Unit:
BARKAN et al.)	
)	Examiner: ...
I.A. Filed: 26 April 1998)	
)	Washington, D.C.
U.S.S.N.: 09/403,897)	
)	February 22, 2000
For: LEPTIN AS AN ...)	

NOTICE OF CHANGE OF CORRESPONDENCE ADDRESS

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please associate this application with customer
number 001444. Our customer number records show that the new
address of Browdy and Neimark, P.L.L.C. is:

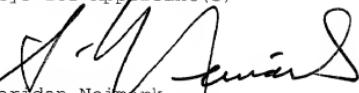
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Our telephone numbers and facsimile numbers remain
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Respectfully submitted,

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